Title page: Lidocaine effects on acetylcholine-elicited currents from mouse superior cervical ganglion neurons

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Abbreviations

ACh, acetylcholine; I_{ACh}, acetylcholine-elicited current; LA, local anaesthetic; LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor; QX-222, 2-(trimethylammonio)-*N*-(2,6-dimethylphenyl) acetamide chloride; QX-314, 2-(triethylammonio)-*N*-(2,6-dimethylphenyl) acetamide bromide; SCG, superior cervical ganglion.

Abstract

Lidocaine is a commonly used local anaesthetic that, besides blocking voltage-dependent Na^+ channels, has multiple inhibitory effects on muscle-type nicotinic acetylcholine (ACh) receptors (nAChRs). In the present study, we have investigated the effects of lidocaine on ACh-elicited currents (I_{ACh}s) from cultured mouse superior cervical ganglion (SCG) neurons, which mainly express heteromeric α3β4 nAChRs. Neurons were voltage-clamped by using the perforated-patch method and I_{ACh}s were elicited by fast application of ACh (100-300 μM), either alone or in presence of lidocaine at different concentrations.

I_{ACh}s were reversibly blocked by lidocaine in a concentration-dependent way (IC_{50} = 41 μM; n_H close to 1) and the inhibition was, at least partially, voltage-dependent, indicating an open-channel blockade. Besides, lidocaine blocked resting (closed) nAChRs, as evidenced by the increased inhibition caused by a 12 s lidocaine application just before its co-application with the agonist, and also enhanced I_{ACh}s desensitisation, at concentrations close to the IC_{50}.

These results indicate that lidocaine has diverse inhibitory actions on neuronal heteromeric nAChRs resembling those previously reported for *Torpedo* (muscle-type) nAChRs (Alberola-Die et al., 2011). The similarity of lidocaine actions on different subtypes of heteromeric nAChRs differs with the specific effects of other compounds, restricted to particular subtypes of nAChRs.

Keywords

Lidocaine, local anaesthetics, nicotinic receptors, open-channel blockade, closed-channel blockade, allosteric modulation, sympathetic neurons

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels (LGICs) that mediate fast synaptic transmission in peripheral and central nervous system. An outstanding characteristic of nAChRs is their wide structural heterogeneity, based in both diverse subunit composition and stoichiometry, which associates to subtype-specific functional and pharmacological properties (Gotti and Clementi, 2004; Dani and Bertrand, 2007). So far, the most studied, and yet the prototypic model for these receptors, is the muscle-type nAChR, localised at the neuromuscular junction of vertebrates and at the electrocytes of some electric fishes, and composed of two α1 subunits and one β1, γ and δ or ε subunits. In the peripheral and central nervous system there are different subtypes of nAChRs, generically called neuronal nAChRs. In mammalian autonomic ganglia neurons, including the superior cervical ganglion (SCG), the predominant nAChR is the heteromeric α3β4 subtype, though there are a significant percentage of combinations with α5 and/or β2 subunits (Gotti et al., 2009; David et al., 2010). Moreover, homomeric α7 receptors are also present, but their currents are only evoked in the presence of PNU 120569 (David et al., 2010). Finally, the two main nAChRs subtypes in the mammalian central nervous system are α4β2 and α7 (Flores et al., 1992; Barrera and Edwardson, 2008) though combinations of α (2-5) with β (2-4) subunits and receptors formed by α9 and/or α10 are also present (Dani and Bertrand, 2007).

Though lidocaine is one of the local anaesthetics (LAs) more commonly used in clinical practice, its detailed mechanisms of action on muscle-type nAChRs have been only recently studied (Alberola-Die et al., 2011); in fact most

studies used permanent-charged lidocaine-analogues such as QX-314 or QX-222 (Neher and Steinbach, 1978; Pascual and Karlin, 1998; Papke et al., 2001). In contrast to QX-314 or QX-222, the molecule of lidocaine has a tertiary amine group, so that, at physiological pH, a fraction of the lidocaine molecules remains uncharged. The simultaneous presence of both forms of lidocaine, charged and neutral, seems to be responsible for the multiple inhibitory actions of lidocaine on muscle-type nAChRs from *Torpedo marmorata* microtransplanted to *Xenopus* oocytes, which include open-channel blockade, enhancement of desensitisation, and blockade of closed-state (resting) receptors (Alberola-Die et al., 2011). The aim of the present study was to characterize in mouse SCG neurons the effects of lidocaine on acetylcholine-elicited currents (I_{ACh}), which are mainly mediated by heteromeric α3β4 nAChRs.

2. Materials and methods

2.1. Animals

Animal handling and experimental procedures were approved by the Spanish Higher Research Council and the University of Vigo Scientific Committee and they conformed to Spanish and European guidelines for the protection of experimental animals (RD1201/2005; 2010/63/UE).

2.2. Cell culture

Mouse SCG cell culture was carried out as described previously (Martínez-Pinna et al., 2002; Romero et al., 2004; Lamas et al., 2009). Briefly, mice (20–60 d of age) were terminally anesthetized with CO_2 and immediately decapitated. SCGs were removed and cleaned up in cold Leibowitz medium (L-15) under a binocular microscope; then, the ganglia were chopped and

incubated in collagenase (2.5 mg/ml in HBSS) for 15 min at 37°C. After washing, the ganglia were then incubated for 30 min in trypsin (1 mg/ml in Hanks). Finally, neurons were mechanically isolated, centrifuged, and plated in 35 mm Petri dishes previously coated with laminin (10 µg/ml in EBSS). Neurons were cultured for 1–2 d at 37°C and 5% CO_2 in L-15 medium containing the following: 24 mM $NaHCO_3$, 10% fetal calf serum, 2 mM L-glutamine, 38 mM D-glucose, 100 UI/ml penicillin, 100 µg/ml streptomycin, and 50 ng/ml nerve growth factor.

2.3. *Perforated-patch whole-cell recording*

Electrophysiological recordings were performed at room temperature and under continuous perfusion (≈10 ml/min) using an Axopatch 200B amplifier (Molecular Devices). Two-step fire-polished patch pipettes, with a tip resistance of 2–4 MΩ, were used to record through perforated patches obtained with amphotericin B (75 µg/ml); patches with series resistance above 20 MΩ were discarded (Rae et al., 1991). Junction potential ≈5 mV was not corrected (Neher, 1992). Data were digitized through a Digidata 1440A and analysed and plotted using the pClamp10 software (Molecular Devices) and Origin 6.1 (OriginLab Corp., Northampton, MA, USA). The sampling frequency was 10 kHz and recordings were low pass filtered at 5 kHz by the amplifier built-in filter. The recording pipette solution contained (in mM): 105 Cs-acetate, 25 CsCl, 3 $MgCl_2$, 0.5 $CaCl_2$, and 20 HEPES, pH=7.2 with Tris (tris(hydroxymethyl)aminomethane). Standard bath solution contained (in mM): 140 NaCl, 3 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 D-glucose, and 10 HEPES, adjusted to pH 7.2 with Tris. Atropine (0.5 µM) and TTX (0.5 µM) were routinely added in order to block muscarinic receptors and voltage-dependent sodium channels,

respectively. Agonists and antagonists were applied using a fast-step perfusion system SF-77B (Warner Instruments, USA).

2.4. Data analysis

To determine the effect of lidocaine on I_{ACh}, we measured I_{ACh} peak evoked by 300 µM ACh alone or together with different lidocaine doses (0.1 µM – 10 mM). I_{ACh}s elicited in the presence of lidocaine were normalised to the I_{ACh} evoked by ACh alone (Control) and data were fitted, using the Origin 6.1 software, to the following form of the Hill equation (1):

$$I/I_{max} = [1 + (IC_{50}/[\text{ACh}])^{nH}]^{-1}$$

where I is the I_{ACh} peak elicited at 300 µM ACh (applied either alone or together with lidocaine), I_{max} is the maximum current recorded, IC_{50} is the lidocaine concentration required to inhibit one-half the maximum current, and n_H is the Hill coefficient.

The rate of desensitisation was determined by measuring the I_{ACh} amplitude elicited by 300 µM ACh, either alone or co-applied with 3 or 30 µM lidocaine, at different times after the current peak. The percentages of desensitisation were obtained using the equation (2):

$$D_{ti} = [1 - (I_{ti}/I_{peak})] \times 100$$

where D_{ti} is the percentage of desensitisation at the specified time, I_{peak} the peak current amplitude, and I_{ti} the current amplitudes remaining 1, 2, and 3 s after the peak.

2.5. Drugs

ACh, atropine sulphate, lidocaine, HEPES, L-15 Leibovitz medium, collagenase, HBSS, trypsin, laminin, EBSS, penicillin, streptomycin and reagents of general use were purchased from Sigma (St. Louis, MO, USA)

2.6. Statistics

The values given in the text and figures correspond to the mean±SEM. When comparing two-group means of normally distributed values, the Student's *t*-test was used. Among-group differences were determined by the Kruskal–Wallis analysis of variance on ranks; the comparison of groups was made using the Dunn's test. A significance level of $P<0.05$ was considered for all cases.

3. Results

3.1. Lidocaine inhibits I_{ACh} in SCG neurons

Cultured SCG neurons were patched and voltage-clamped at -60 mV, unless otherwise stated, by using the perforated-patch whole cell recording. For each experimental condition, recordings were made from neurons of 5-8 independent cell cultures, unless otherwise stated. Fast application of ACh (300 µM) for 5 s to a clamped neuron elicited a desensitising inward current (Lamas et al., 1997), which was due to the activation of nAChRs expressed by these neurons (Figure 1A, Control). When ACh (300 µM) was co-applied with different doses of lidocaine (0.1 µM to 10 mM), to the same neuron, the I_{ACh} peak decreased in a dose-dependent way (Figure 1A). Similar results were obtained in 7 different cells. The average dose-inhibition relationship (Figure 1B) was well fitted to a single sigmoid curve, from which could be estimated an IC_{50} of 41 µM and a slope (n_H) of 0.90 ± 0.04. These results are in good agreement with those reported by Gentry and Lukas (2001) by measuring $^{86}Rb^+$ efflux in SH-SY5Y

cells expressing α3β4 nAChRs (IC_{50} = 63; n_H = 1.2), in spite of the methodological differences.

3.2. Lidocaine effects on I_{ACh} desensitisation

Besides decreasing I_{ACh} peak, lidocaine modified the I_{ACh} inactivation kinetics of SCG neurons, and this effect was dependent on the lidocaine dose, as previously described for *Torpedo* nAChRs microtransplanted to *Xenopus* oocytes (Alberola-Die et al., 2011). Thus, co-application of a low dose of lidocaine (3 µM, which caused about 10% inhibition of I_{ACh}) with ACh (300 µM) did not affect I_{ACh} desensitisation (D_{ti} values at 1, 2, and 3 s were: 34±5%, 63±7%, and 76±6%, n=9, for ACh alone versus 41±8%, 65±8%, 77±7%, n=7, for ACh plus 3 µM lidocaine (p>0.05, ANOVA on ranks); see Figure 2A, B). However, higher doses of lidocaine (30 µM, close to the IC_{50}), in the presence of ACh (300 µM), significantly accelerated I_{ACh} decay (D_{ti} values at 1, 2, and 3 s were: 68±6%, 86±3%, 93±2%, n=7 (p<0.05, ANOVA on ranks); see Figure 2A, B).

3.3. *Lidocaine inhibition as a function of the membrane potential*

To determine whether, or not, lidocaine blockade of I_{ACh} was voltage-dependent, I_{ACh}s were elicited by applying ACh (100 µM) either alone (Figure $3A_1$) or co-applied with lidocaine (100 µM; Figure $3A_2$), at different membrane potentials (from -80 to +20 mV, in steps of 20 mV). The current-voltage relationship (*i/v*) for the I_{ACh}s evoked by ACh alone (n=9), showed a reversal potential close to 0 mV and marked inward rectification (Figure $3A_1$, B, closed circles), similar to that described for nAChRs of rat sympathetic ganglion neurons (Lamas et al., 1997; Mathie et al., 1990). When lidocaine was co-

applied with ACh (n=7) I_{ACh} reversal potential was not affected, but I_{ACh} amplitude decreased and the extent of inhibition depended on the membrane potential. Thus, a greater blockade was observed at more hyperpolarized potentials (Figure 3A_2, B, open circles). This voltage-dependence of I_{ACh} inhibition by lidocaine is better shown by plotting the fraction of the I_{ACh} left by lidocaine versus membrane potential, which evidences, at negative potentials, a lineal function (Figure 3C). The voltage dependence could not be properly assessed at positive potentials, because of the marked inward rectification of I_{ACh} at these potentials (Figure 3B). Nevertheless, the value of fractional I_{ACh} left by lidocaine at 0 mV, estimated from the fitted line, was roughly 50%, indicating that still at this membrane potential there is an important blockade of nAChRs. This suggesting that lidocaine exerts also a voltage-independent blockade, as it has been previously reported for muscle-type nAChRs (Alberola-Die et al., 2011) and for other related LAs, such as procaine (Adams, 1977).

3.4. *Lidocaine blockade of closed-state (resting) nAChRs*

Given that lidocaine causes voltage-independent inhibition of I_{ACh} in SCG neurons, we wanted to know whether this particular blockade of nAChRs was due to the binding of lidocaine to closed-state receptors, as we previously reported for muscle-type nAChRs (Alberola-Die et al., 2011). We compared the degree of I_{ACh} inhibition obtained by pre-applying lidocaine (100 µM) for 12 s, and subsequently co-applying ACh (300 µM) plus lidocaine (100 µM) with that evoked by direct co-application of ACh and lidocaine, at the same doses. When lidocaine was pre-applied to a neuron before its co-application with ACh, the extent of I_{ACh} inhibition was much higher than that evoked, in the same neuron, by solely co-application of lidocaine and ACh (Figure 4A_1 and A_2). On average,

the percentage of I_{ACh} blockade reached 93±1% (n=5, from 3 independent cell cultures) and 65±3% (n=6; $p<0.05$, *t*-test), for responses evoked with and without lidocaine pre-application, respectively. This enhanced I_{ACh} inhibition elicited by lidocaine pre-application can be explained assuming that lidocaine blocks closed-state nAChRs, and so, precludes their opening when lidocaine is subsequently co-applied with ACh (Alberola-Die et al., 2011).

4. Discussion

These results show that lidocaine mechanisms of action on neuronal heteromeric nAChR present in the peripheral nervous system are similar to those previously reported by Alberola-Die et al. (2011) for muscle-type nAChRs. The similarity of the actions evoked by lidocaine in dissociated SCG neurons and in oocytes microtransplanted with muscle-type nAChR has two relevant implications: **i)** It indicates that lidocaine has similar modulating actions on different heteromeric nAChRs, including muscle-type and α3β4 nAChRs, which is the main subtype in sympathetic neurons; but also most likely over α3β4α5 and α3β4β2, which together constitute almost half the number of nAChRs in the mouse SCG neurons (David et al., 2010). This being of particular interest because many molecules acting on nAChRs have marked specificity on particular subtypes. So, for instance, whereas BW284c51, a quaternary-ammonium cholinesterase inhibitor, is a powerful inhibitor of muscle-type nAChRs (Olivera-Bravo et al., 2005, 2007) it barely blocks either heteromeric α4β2 or homomeric α7 nAChRs (Fayuk and Yakel, 2004). Similarly, niflumic and flufenamic acids inhibit α3β2 but potentiate α3β4 nAChRs (Zwart et al., 1995). **ii)** The effects evoked by lidocaine on nAChRs in either SCG neurons or muscle fibres have some similarities to those described for other members of

the Cys-loop family of receptors. So, lidocaine inhibits, though at higher concentrations, glycine and $GABA_A$ receptors expressed in oocytes (Hara and Sata, 2007), although its mechanisms of blockade remain to be elucidated. Furthermore, it has been recently shown that lidocaine, and other quaternary ammonium compounds, also blocks prokaryotic pentameric ligand-gated channels (GLIC, protein from *Gloeobacter violaceous*), by its binding within the channel pore, causing a voltage-dependent blockade (Hilf et al., 2010), which is something similar to that we found for neuronal nAChRs from SCG neurons. Nevertheless, from our results, lidocaine must also bind to other regions of heteromeric nAChRs, since it also caused closed-channel blockade and changes in desensitisation, which cannot be explained by a single binding site in heteromeric nAChRs.

It should be emphasized that lidocaine is a compound widely used in clinical practice as LA and also as antiarrhythmic drug (class I) (Sheets et al., 2010). For nerve blockade therapies, or for epidural or spinal anaesthesia, lidocaine is commonly administered at doses of 0.5-5% (20-200 mM) (Covino and Wildsmith, 1998), reaching the target area at concentrations in the millimolar range, to warrant an effective blockade of voltage-dependent Na^+ channels. Thus, at these concentrations, lidocaine will have relevant direct blocking actions on nAChRs of muscle fibers and vegetative ganglia, given that their IC_{50} is far below 1 mM.

In conclusion, these results confirm and extend previous studies on the mechanisms of modulation of neuronal nAChRs by lidocaine, providing new evidences supporting that neuronal heteromeric nAChRs expressed in sympathetic neurons are strongly modulated by lidocaine, which inhibits these

receptors by different and independent ways. It remains to be known whether heteromeric nAChR in the central nervous system, mainly of the α4β2 subtype, and the homomeric α7 nAChRs, are similarly modulated by lidocaine or other LAs.

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Conflict of interest and source of founding

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References

Adams, P.R., 1997. Voltage jump analysis of procaine action at frog end-plate. J. Physiol. 268, 291-318.

Alberola-Die, A., Martinez-Pinna, J., González-Ros, J.M., Ivorra, I., Morales, A., 2011. Multiple inhibitory actions of lidocaine on *Torpedo* nicotinic acetylcholine receptors transplanted to *Xenopus* oocytes. J. Neurochem. 117, 1009-1019.

Barrera, N.P., Edwardson, J.M., 2008. The subunit arrangement and assembly of ionotropic receptors. Trends Neurosci. 31, 569-576.

Covino, B.G., Wildsmith, J.A.W., 1998. Clinical pharmacology of local anesthetic agents. In: M.J. Cousins, P.O. Bridenbaugh (Eds.), Neural blockade in clinical anesthesia and management of pain, Lippincott-Raven, Philadelphia, pp. 97-128.

Dani, J.A., Bertrand, D., 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 47, 699-729.

David, R., Ciuraszkiewicz, A., Simeone, X., Orr-Urtreger, A., Papke, R.L., McIntosh, J.M., Huck, S., Scholze, P., 2010. Biochemical and functional properties of distinct nicotinic acetylcholine receptors in the superior cervical ganglion of mice with targeted deletions of nAChR subunit genes. Eur. J. Neurosci. 31, 978-993.

Fayuk, D., Yakel, J.L., 2004. Regulation of nicotinic acetylcholine receptor channel function by acetylcholinesterase inhibitors in rat hippocampal CA1 interneurons. Mol. Pharmacol. 66, 658-666.

Flores, C.M., Rogers, S.W., Pabreza, L.A., Wolfe, B.B., Kellar, K.J., 1992. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. Mol. Pharmacol. 41, 31-37.

Gentry, C.L., Lukas, R.J., 2001. Local anesthetics noncompetitively inhibit function of four distinct nicotinic acetylcholine receptor subtypes. J. Pharmacol. Exp. Ther. 299, 1038-1048.

Gotti, C., Clementi, F., 2004. Neuronal nicotinic receptors: from structure to pathology. Prog. Neurobiol. 74, 363-396.

Gotti, C., Clementi, F., Fornari, A., Gaimarri, A., Guiducci, S., Manfredi, I., Moretti, M., Pedrazzi, P., Pucci, L., Zoli, M., 2009. Structural and functional diversity of native brain neuronal nicotinic receptors. Biochem. Pharmacol. 78, 703-711.

Hara, K., Sata, T., 2007. The effects of the local anesthetics lidocaine and procaine on glycine and γ-aminobutiric acid receptors expressed in *Xenopus* oocytes. Anesth. Analg. 104, 1434-1439.

Hilf, R.J., Bertozzi, C., Zimmermann, I., Reiter, A., Trauner, D., Dutzler, R., 2010. Structural basis of open channel block in a prokaryotic pentameric ligand-gated ion channel. Nat. Struct. Mol. Biol. 17, 1330-1336.

Lamas, J.A., Romero, M., Reboreda, A., Sánchez, E., Ribeiro, S.J., 2009. A riluzole and valproate-sensitive persistent sodium current contributes to the resting membrane potential and increases the excitability of sympathetic neurons. Pflügers Archiv 458, 589-599.

Lamas, J.A., Selyanko, A.A., Brown, D.A., 1997. Effects of a cognition-enhancer, linopirdine (DuP 996), on M-type potassium currents ($I_{K(M)}$) and some other voltage- and ligand-gated membrane currents in rat sympathetic neurons. Eur. J. Neurosci. 9, 605-616.

Martínez-Pinna, J., Lamas, J.A., Gallego, R., 2002. Calcium current components in intact and dissociated adult mouse sympathetic neurons. Brain Res. 951, 227-236.

Mathie, A., Colquhoun, D., Cull-Candy, S.G., 1990. Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion neurons. J. Physiol. 427, 625-655.

Neher, E., Steinbach, J.H., 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. J. Physiol. 277, 153-176.

Neher, E., 1992. Correction for liquid junction potentials in patch clamp experiments. Methods Enzymol. 207, 123-31.

Olivera-Bravo, S., Ivorra*,* I., Morales, A., 2005. The acetylcholinesterase inhibitor BW284c51 is a potent blocker of *Torpedo* nicotinic AchRs incorporated into the *Xenopus* oocyte membrane. Br. J. Pharmacol. 144, 88-97.

Olivera-Bravo, S., Ivorra, I., Morales, A., 2007. Diverse inhibitory actions of quaternary ammonium cholinesterase inhibitors on Torpedo nicotinic ACh receptors transplanted to Xenopus oocytes. Br. J. Pharmacol. 151, 1280-1292.

Papke, R.L., Horenstein, B.A., Placzek, A.N., 2001. Inhibition of wild-type and mutant neuronal nicotinc acetylcholine receptors by local anesthetics. Mol. Pharmacol. 60, 1365-1374.

Pascual, J.M., Karlin, A., 1998. Delimiting the binding site for quaternary ammonium lidocaine derivatives in the acetylcholine receptor channel. J. Gen. Physiol. 112, 611-621.

Rae, J., Cooper, K., Gates, P., Watsky, M., 1991. Low access resistance perforated patch recordings using amphotericin B. J. Neurosci. Methods 37, 15–26.

Romero, M., Reboreda, A., Sánchez, E., Lamas, J.A., 2004. Newly developed blockers of the M-current do not reduce spike frequency adaptation in cultured mouse sympathetic neurons. Eur. J. Neurosci. 19, 2693-2702.

Sheets, M.F., Fozzard, H.A., Lipkind, G.M., Hanck, D.A., 2010. Sodium channel molecular conformations and antiarrhythmic drug affinity. Trends Cardiovasc. Med. 20, 16-21.

Zwart, R., Oortgiesen, M., Vijverberg, H.P., 1995. Differential modulation of alpha 3 beta 2 and alpha 3 beta 4 neuronal nicotinic receptors expressed in Xenopus oocytes by flufenamic acid and niflumic acid. J. Neurosci. 15, 2168-2178.

FIGURE LEGENDS:

Figure 1. *Lidocaine inhibits I_{ACh}s in SCG neurons.* (**A**) Superimposed recordings of I_{ACh}s obtained in the same SCG neuron by applying, for 5 s, ACh either alone (300 µM, Control) or with the indicated doses of lidocaine (+ 3, 10, 30, 100, 300 or 1000 µM Lid). In this and following figures, the bar above the records indicates the application span, and the holding potential was -60 mV, unless otherwise stated. I_{ACh}s were obtained at intervals of 5 min, in order to minimize desensitisation effects. (**B**) Dose-I_{ACh} inhibition relationship for lidocaine. I_{ACh}s evoked in the presence of lidocaine were normalised to the I_{ACh} elicited by ACh alone. Each point corresponds to the mean±S.E.M. of 5-7 different cells and solid line represents the fitting to a sigmoid curve (see equation 1, Materials and methods).

Figure 2. *Lidocaine effects on I_{ACh} desensitisation*. (**A**) Superimposed I_{ACh} records elicited by ACh (300 µM) applied either alone (Control, black) or plus lidocaine at two different doses (3 or 30 µM, red). Records are normalised to the same size in order to better compare the inactivation kinetics. (**B**) Graph showing the percentage of I_{ACh} desensitisation at different times (1, 2, and 3 s) after I_{ACh} peak, when neurons were superfused with ACh (300 µM) alone (closed circles and black line, n=9), or co-applied with either 3 µM lidocaine (open red circles and red line, n=7) or 30 µM lidocaine (open red triangles and red line, n=7). Data are the mean±S.E.M. and asterisks indicate significant differences with the control values (p<0.05, ANOVA on ranks).

Figure 3. *Lidocaine blocks nAChRs in a voltage-dependent manner*. (**A_1** and **A_2**) Superimposed recordings of I_{ACh}s evoked, in the same neuron, by 100 µM ACh either alone (**A_1**) or co-applied with 100 µM lidocaine (**A_2**), while clampling the cell at different holding potentials (values on the right, in mV). (**B**) *i/v* relationships of the I_{Ach}*s* elicited at each voltage, (normalised to the I_{ACh} evoked by ACh alone at -60 mV; each point is the average±S.E.M. of 5-9 neurons), in presence of ACh alone (100 µM, filled circles) or co-applied with lidocaine (100 µM, open circles). For some points, the error bar is within the symbol. (**C**) Plot of the fraction of the I_{Ach} left by lidocaine ($I_{ACh+Lid}$), normalised to the control I_{ACh}, against the membrane potential, showing the voltage-dependent blockade of nAChRs by lidocaine. Each data is the mean±S.E.M. of, at least, 5 cells.

Figure 4. *Inhibition of nAChRs by lidocaine pre-application*. (**A_1**) Representative I_{ACh}s records elicited, in the same neuron, by 300 µM ACh either alone (Control, black record), or plus 100 µM lidocaine after pre-applying lidocaine, at the same concentration, for 12 s (+ 100 µM Lid, red recording). (**A_2**) I_{ACh}s evoked by 300 µM ACh alone (Control, black record) or co-applied with 100 µM lidocaine (+ 100 µM Lid, red recording), without lidocaine pre-application. (**B**) Column graph showing the percentage of I_{ACh} inhibition when 300 µM ACh and 100 µM lidocaine were co-applied with (n=5) or without (n=6) a 12 s lidocaine pre-application. Data are the mean±S.E.M. and the asterisk indicates significant differences between groups (p<0.05, *t*-test).